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Advanced technology-driven therapeutic interventions for prevention of tendon adhesion: design, intrinsic and extrinsic factor considerations

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Abstract

Tendon adhesion formation describes the development of fibrotic tissue between the tendon and its surrounding tissues, which commonly occurs as a reaction to injury or surgery. Its impact on function and quality of life varies from negligible to severely disabling, depending on the affected area and extent of adhesion formed. Thus far, treatment options remain limited with prophylactic anti-inflammatory medications and revision surgeries constituting the only tools within the doctors' armamentarium - neither of which provides reliable outcomes. In this review, the authors aim to collate the current understanding of the pathophysiological mechanisms underlying tendon adhesion formation, highlighting the significant role ascribed to the inflammatory cascade in accelerating adhesion formation. The bulk of this article will then be dedicated to critically appraising different therapeutic structures like nanoparticles, hydrogels and fibrous membranes fabricated by various cutting-edge technologies for adhesion formation prophylaxis. Emphasis will be placed on the role of the fibrous membranes, their ability to act as drug delivery vehicles as well as the combination with other therapeutic structures (e.g., hydrogel or nanoparticles) or fabrication technologies (e.g., weaving or braiding). Finally, the authors will provide an opinion as to the future direction of the prevention of tendon adhesion formation in view of scaffold structure and function designs.

Keywords: tendon adhesion; fibrous membranes; drug delivery; growth factors; nanoparticles.

1. Introduction

Tendons are a dense connective tissue connecting muscle to bone, thereby transmitting forces across a joint to produce motion [1]. Despite exhibiting high tensile strengths capable of withstanding large forces generated by skeletal muscle contraction, tendons are susceptible to damages ranging from chronic overuse tendinopathies to spontaneous or trauma-related rupture [2]. According to previous reports, each year more than 30 million people worldwide suffer from tendon injuries with an estimated healthcare cost of over $140 billion [3]. Overall, nearly 25% of adults are expected to be affected by tendon-related injuries due to the population's increasing life expectancy and associated degeneration of all aspects of the human body [1, 4]. To add insult to injury, currently available therapies are both invasive and fraught with complications, of which tendon adhesion (TA) formation remains amongst the principally debilitating ones [5]. Once injured and regardless of the cause, all tendon injuries heal suboptimally due to the formation of disordered scar tissue [6]. Such restrictive adhesions within the tendons' synovial sheaths result in impaired functionality and an increased risk of re-rupture, significantly hampering the tendon healing outcomes [7].

Previous approaches to prevent TA formation dating back to the 1980s included the use of nonsteroidal anti-inflammatory drug (NSAIDs), such as ibuprofen, which when injected into the injured area reduces expressions of pro-inflammatory factors such as arachidonic acid, which is thought to decrease post-traumatic endogenous local damage [8]. However, the excess use of NSAIDs may cause detrimental side effects associated with cardiovascular or genitourinary systems [9]. To overcome these issues, various biologicals like growth factors (GFs, e.g., vascular endothelial growth factor (VEGF) and fibroblast growth factor-β (FGF-β)) and genes (e.g., nucleic acids, microRNA (miRNA), small interfering RNA (siRNA)) have been applied to controlling cell fate and functions at the delivery site by modulating signal transduction [10]. Although the direct delivery of these biologicals showed good therapeutic potential *in vivo*, there are still many limitations for their successful clinical use. For example, due to the fast inactivation and clearance in the local microenvironment, the continuous supplementation of exogenous GFs is needed, which may increase the treatment costs and the risk of local adverse effects. Although gene therapy can be an alternative approach for GF therapy to achieving controlled long-term protein expression *in situ*, it faces many challenges like low *in vivo* cellular uptake efficiency and the natural instability. Most importantly, topical drug delivery can only control cell behaviors to some extent, and thus spatial isolation of tendon damaged site from the external synovial sheath

by application of physical barriers has become a feasible strategy to prevent the invasion of external fibroblasts and the later adhesion formation. Currently, physical barriers made of non-degradable materials such as silica gel and gold foil have been trialed to prevent TA [11]. Degradable alternatives like human-derived fibrin sealant were shown to reduce TA formation in the early stages of healing in a rabbit tendon injury model by inhibiting fibrous tissue formation [12]. Their degradation, however, resulted in an inflammatory reaction and loss of said barrier, resulting in no long-term difference in range of motion.

It has become evident that neither GF application nor physical barrier formation by themselves resulted in clinically relevant attenuation in TA formation. Therefore, current studies shift their focus to a combinatory approach, using porous 3D barrier scaffolds capable of being functionalized or loaded with different pharmaceuticals and/or biologicals to accelerate tendon healing whilst combatting TA formation. These scaffolds were fabricated through various technologies including conventional techniques like freeze-drying, gas foaming, solvent casting, simple knitting, and braiding [13]. Despite being potential, these technologies fail to recapitulate the subtle fibrous structure of native tendon extracellular matrix (ECM) [14]. In recent years, different fiber-based technologies have been investigated for the fabrication of fibrous scaffolds such as electrospinning, wet spinning, jet spinning, 3D printing and melt electrowriting [13]. Although all of them could generate fiber-based scaffolds, the low productivity of wet spinning, heterogeneous fiber diameter of jet spinning, uneven scaffold thickness of melt electrowriting, high-cost of 3D printing curbed their application in scaffold fabrication. To this end, electrospinning is the most commonly adapted technique for constructing fibrous membranes due to (1) their functional resemblance to tendon sheaths in acting as physical barriers, (2) ECM-like microstructure rendering them highly porous with a large surface, (3) tunable architecture parameters (i.e., fiber alignment and diameter), mechanical properties and degradation rates, and (4) the ability to modulate local inflammatory reactions via controlled release of integrated cell signaling factors [15, 16].

The goal of this review is to provide a comprehensive, yet succinct discussion of the status quo of various therapeutic intervention strategies for TA prevention, with an emphasis on the fibrous barrier membranes fabricated by the state-of-the-art electrospinning technology. The remainder of the review first critically appraise the current understanding of the pathophysiology underlying TA formation with regards to the inflammatory response, the role of fibroblasts and the influence of GFs. Then, we delineate in depth how to integrate the

pharmacological agents or biomolecules with different therapeutic structures like nanoparticles (NPs), hydrogels and fibrous membranes, and discuss in detail how to combine these therapeutic structures or fabrication technologies for construction of higher-performance therapeutic platforms (**Figure 1**). Furthermore, we highlight several outstanding questions pertaining to the future of therapeutic platform design in an effort to explore new avenues for the prophylaxis and treatment of TA.

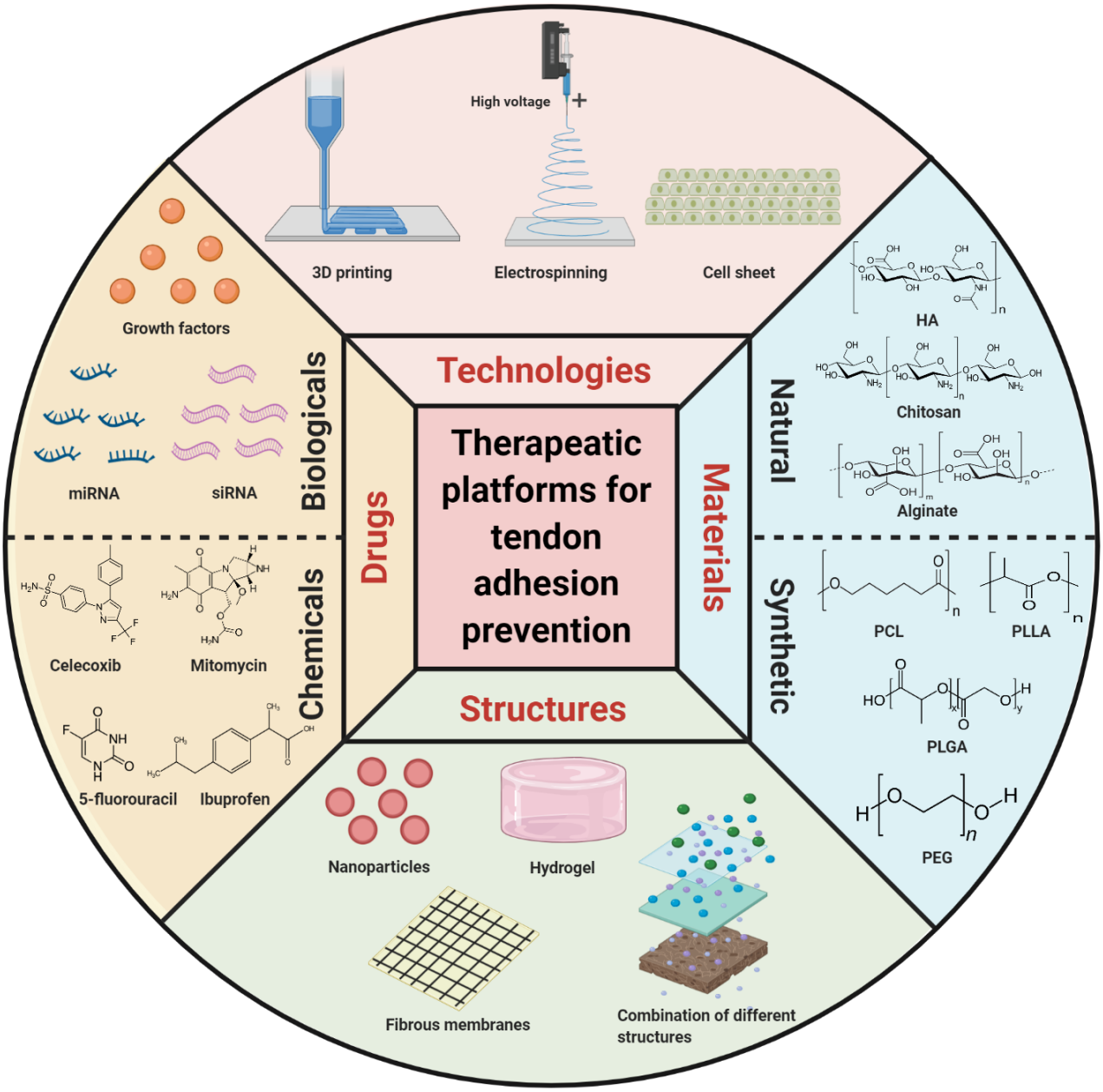

Figure 1. Basic design considerations of therapeutic platforms for prevention of tendon adhesion including fabrication technologies, scaffold materials, therapeutic structures and loaded drugs. Figure created with BioRender.com.

2. Tendon healing and adhesion mechanism

Due to low cellularity and poor vascularity, the healing process of tendon injury is relatively

slow (more than 1 year) as compared to other musculoskeletal tissues [17]. In addition, during the repair stage, peritendinous adhesion, one of the most severe complications, can be formed within the rupture site, impeding the tendon glide throughout flexion and decreasing the mechanical properties of repaired tendons [18, 19]. Currently, TA is thought to be caused by the imbalance generated between the intrinsic and extrinsic healing pathways comprised of tenocytes and fibroblasts, respectively (**Figure 2A**) [19, 20]. Intrinsic healing occurs when tenocytes from epitenon and endotenon proliferate and migrate to the injury site, while extrinsic healing is caused by penetration of fibroblasts from the surrounding sheath and synovium. Both intrinsic and extrinsic healing are part of normal tendon regeneration. However, due to the poor cell density and GF activity, the tendon inherently has limited capacity for intrinsic healing. On the other hand, infiltrating fibroblastic activity usually results in adhesion formation via collagen and fibrin deposition between the injury site and the tendon sheath, thereby significantly reducing the range of motion of the affected joint [21]. In addition, inflammatory exudates further exacerbate fibrin leakage and thus adhesion formation [5]. Here, we concluded some important factors in the regeneration process including the inflammatory response, cell behaviors, and GFs/genes that could influence either the intrinsic or extrinsic healing pathway and thus delicately tune the imbalance.

In classic view of the tendon repairing, the healing process present remarkable time-dependent behaviors and can be divided into three overlapping phases tuned by different cell types, cytokines and GFs (**Figure 2B**) [22]. The first phase mainly consists of the inflammatory activities including the inflammatory cells and exogenous fibroblasts invading into the injury site. The second phase is a repairing process composed of fibroblast proliferation, excessive ECM synthesis, activation and tenogenic differentiation of tendon stem/progenitor cells (TSPCs), extensive vascular and nerve outgrowth. In the final remodeling phase, the newly produced collagen is rearranged to form the cured tissue [4]. The intrinsic compartment exhibits limited metabolic rate and reparative capacity in the early two stages due to the insufficient nutrients. Thus, the extrinsic healing dominates the first two stages of tendon repair controlled by cells migrating from the peripheral tissues. Ideally, with the proceeding of repairing, the intrinsic healing will gradually dominate the healing process resulting in good biomechanics for regenerated tendon with fewer complications. However, the tendon injury is often accompanied by the destruction of tendon sheath structure, which will tip the dynamic balance of the extrinsic and intrinsic compartment with time, further increasing the complexity of the healing process and the possibility of TA

formation [19, 23].

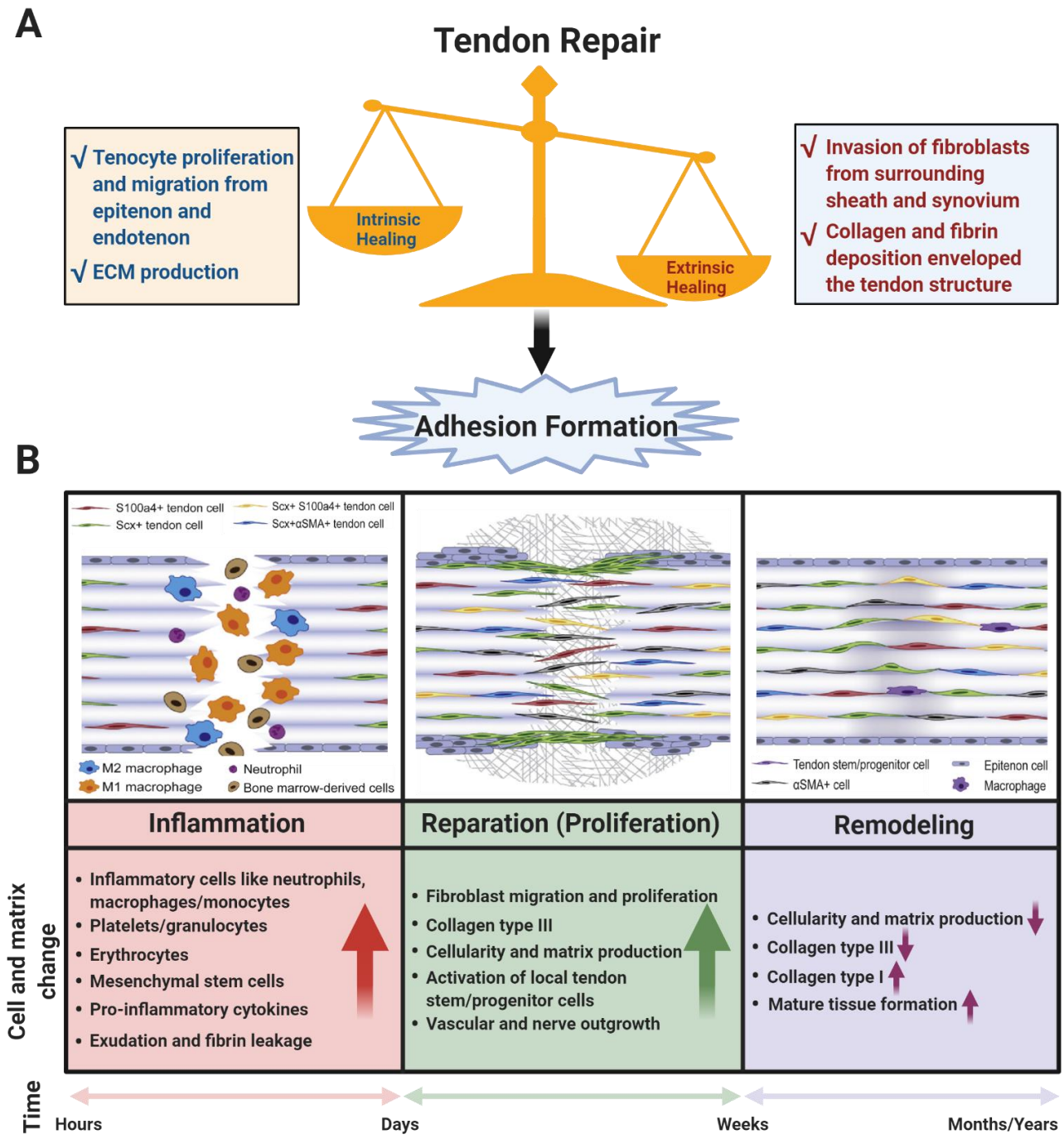

Figure 2. (A) Tendon adhesion formation due to the unbalance between the intrinsic and extrinsic wound healing. (B) The key cell/matrix changes and duration of three tendon injury repair phases (inflammation, reparation, and remodeling). Scx, S100a4, αSMA refers to the scleraxis, calcium binding protein, alpha smooth muscle actin, respectively. Reproduced from ref. [22] with permission from Elsevier. Figure created with BioRender.com.

3. Factors affecting tendon adhesion

3.1 Inflammatory response

Following tendon rupture, an acute inflammation response to injury is initiated and will last

for 3 to 7 days [24]. Within 2-3 days, the gene expression level of pro-inflammatory cytokines at the defect site is increased by several thousand times and is accompanied by the infiltration of circulating inflammatory cells like macrophages, monocytes and neutrophils [22]. This cellular response after tendon injury often increases the risk of TA formation and disorganized matrix degradation, both of which are detrimental to tendon healing [25, 26]. The underlying mechanism is thought to be the significant up-regulation of matrix degradation and inflammation-related factors by the activated fibroblasts, as demonstrated by a down-regulation of ECM-stimulating factors when exposed to inflammatory factors [27, 28]. The local inflammation in tendon defect site can also lead to increased exudation, aggravating the fibrin leakage and promoting adhesion formation. Therefore, understanding and adjusting the inflammatory response during the healing phases will be crucial in reducing the occurrence of TA.

3.2 Cell behaviors

Next, both extrinsic cells from peritendinous soft tissue and intrinsic cells from the epitenon and endotenon migrate and proliferate in the injured site and then form the granulation tissue locally [22, 24]. Among them, fibroblasts, the resident cellular constituents of tendon sheath and synovium, secrete large amount of collagenous materials in the area of tendon injury after activation, which can contribute to adhesion formation between the healing tendon and surrounding tissues [29]. The resultant matrix contains chemotactic components, which further stimulates fibroblast migration and proliferation [30]. This synergistic interaction results in further aggravation of TA. Therefore, conventional strategy by placing a physical barrier between tendon and soft tissue could reduce the adhesion formation to some extent by blocking the infiltration and proliferation of fibroblasts. Meanwhile, exogenously supplementing some reparative cells like tenocytes, mesenchymal stem cells (MSCs), and tendon stem / progenitor cells (TSPCs) has also been proposed to enhance the intrinsic healing [31-33]. Notably, due to the superior self-renewal and multipotency as well as the important role in maintaining tendon homeostasis, TSPCs has been combined with different biomaterials or biological cues to augment tendon healing, showing great promise in stem cell-based tendon tissue engineering [34].

3.3 Growth factors and genes

GFs have been extensively studied in the TA formation process, considering their significant roles in the cell recruitment and ECM deposition affecting the tendon physiology and healing after injury. Specifically, GFs such as transforming GF-β (TGF-β), which are responsible for

regulating fibroblast proliferation, can affect the formation of TA positively or negatively [35]. TGF-β is a multifunctional cytokine that belongs to the transforming GF superfamily. Among TGF-β, TGF-β1, an isoform of TGF-β, was found to induce fibrotic changes and adhesion formation in healing tendon tissues [36]. It was observed that TGF-β1 upregulation during the early phases of tendon healing was associated with increased deposition of type I and III collagen and fibronectin corroborating that tendon healing by scar tissue formation underlies TA formation [37-39]. Blocking TGF-β1 by delivery of miRNA has been showed to downregulate type III collagen gene expression and thus decrease adhesion *in vivo* [40]. However, contradictory evidence indicated the downregulation of TGF-β1 might also reduce the mechanical strength of repaired tendon[39].

VEGF and FGF-β were shown by themselves and in combination to enhance tendon healing by promoting mesenchymal stem cell (MSC) proliferation and differentiation towards tenogenic lineages, increasing vascularization and enhancing mechanical strength of the regenerating tendon [41, 42]. For this reason, simultaneous delivery of TGF-β1 and VEGF and/or FGF-β has been put forward to decrease the peritendinous adhesion without sacrificing tendon strength [43]. Platelet derived growth factor (PDGF) has also been shown to enhance tendon repair by increasing angiogenesis and stimulating tenoblast migration into the injury site and promoting differentiation into tenocytes [44]. Both bone morphogenic protein (BMP)-12 and BMP-14 induced tenogenic differentiation of MSCs [45], with BMP-14 having been shown to exhibit the additional benefit of reducing adhesion formation during tendon healing [35]. With infinite numbers of GFs and GF combinations, the ideal therapeutic dosing regimen to obtain tendon healing without additional pro-fibrotic effects remains elusive. To this end, a naturally occurring cocktail of GFs-platelet rich plasma (PRP) has come under intense investigation [46]. Several studies investigating the treatment of equine tendon injuries demonstrate improved metabolic activity and superior mechanical strengths of tendons supplemented with PRP [47, 48]. A study conducted by Johnson et al. investigating the impact of PRP on TA formation concluded that there was no difference in adhesion formation between the control and study tendons [49]. Several important limiting factors of PRP treatment were raised including the high cost, the inability to reliably quantify and consistently deliver the same GF concentrations. The authors argue that another limitation lies in the potential for any GF containing solution to diffuse away from the intended target site, rendering both the effective concentration and potential downstream side effect (i.e., ectopic tissue formation and tumor development). To solve these problems, the alternate materials-based strategies have been put forward by many researchers to impart

the biological needs of these materials [14]. For instance, a potential GF- and gene- free strategy to improve the therapeutic effect of these scaffolds for tendon repair could involve forming an organic-inorganic composite that can sustainably release biologically relevant components like silicon, copper ions or amino acids by *in vivo* scaffold degradation [14, 50]. These released components can then participate in different signaling pathways independently or cooperatively to regulate cell functions and better facilitate tissue healing. In addition, the release profiles of these components into the tendon milieu could be easily controlled by appropriate scaffold structure design (e.g., incorporation of slowly biodegradable inorganic nanoparticles or formation of an organic-inorganic multi-network structure) [51]. Most importantly, such biological-free strategy allows for largescale preparation and represents a ready-to-use therapeutic approach, possessing a great potential for clinical translation.

3.4. Signaling pathways

Although the above three factors provide insight into the mechanism of TA formation during healing, these factors may rely on specific signaling transduction process at the molecular level to convey their effects into the cells. After an acute injury or chronic damage, the tendon undergoes biomechanical changes such as loss of collagen fiber tension, primary cilium deformation and nuclear deformation [37]. These mechanical changes result in level changes of the messenger molecules (e.g., ions or cytokines). When the change in messenger level accumulate to a certain threshold, some pathological reaction such as inflammation may be raised by a certain signal transduction pathway [37]. For example, the nuclear factor-kappa B (NF-κB) pathway is proved to be involved in the initial stage of inflammation in the tendon repair process [52]. The pathway includes a cascade of kinases, including NIK (NF-κB inducing kinase) and IKK (IκB kinase), with the downstream product of NF-κB. NF-κB is the common transcription factor for many inflammatory factors related genes such as Interleukin 1β (IL-1β), tumor necrosis factor alpha (TNF-α) [53]. Thus, many cytokines and GFs acting in this pathway may play crucial role in TA formation [52]. Abraham et al. has found that inhibition of IKKβ in the NF-κB pathway could mitigate tendinopathy, potentially TA, by blocking the entire pathway [54]. Besides, inhibition of the transcription factor RelA/p65, an important subunit in the NF-κB complex, could prevent TA by down regulation of the NF-κB pathway [52].

The behaviors of fibroblasts could be modulated by a series of pathways such as the mitogen-activated protein kinases (MAPKs) pathways. It is a class of pathway to control

gene expression and cell proliferation [55]. It has been found that the extracellular signal-regulated kinase (ERK) 1 and 2 belongs to this class of pathway and play a crucial role in the regulation of exogenous fibroblast proliferation and collagen synthesis, which are the prerequisites of TA formation. Inhibiting the gene or downregulating the phosphorylation of the protein has successfully prevented TA [18].

Besides, TGF-β is also highly related to the TA formation and the TGF-β signaling pathway could work in conjunction with other pathways like the MAPK pathway and the BMP pathway to perform tasks like collagen synthesis and tissue remodeling [56]. Smads protein 2 and 3 (SMAD2/3) is a molecule that acts as a transcription factor of the TGF-β as well as a signal transducer in the TGF-β pathway [57]. Upregulating SMAD2/3 phosphorylation and SIRT1 (Sirtuin 1) could stimulate TGF-β expression in fibroblasts to secrete more ECM and thus lead to more severe adhesion [18]. Blocking the pathways of TGF-β1 transducing may represent a useful way to circumvent TA formation and increase range of motion in flexor tendons following injury [58]. Alayna E. Loiselle et al. inhibited SMAD3 by antisense oligonucleotides (ASO) as an effective strategy to alleviate adhesion by suppressing TGF-β1 signaling [57]. Similarly, down regulation of connective tissue growth factor (CTGF), a matricellular protein in the TGF-β1 pathway, could suppress the fibroblast proliferation and ECM excretion during the early stage of tendon healing to prevent scarring during tendon repair [59]. Altogether, it should be noted that TA is a complex process that involves multiple signaling pathways interacting with each other. Although regulation of some signaling pathways has showed to be effective in TA prevention, how exactly these pathways interact is not yet fully understood [37].

4. Advanced technology-driven therapeutic interventions for TA prevention

4.1 Nanoparticles (NPs)

NPs are widely used as delivery vehicles to provide protection for vulnerable cargos such as pharmacological agents, GFs and genes. Compared with large particles, NPs are internalized by cells more easily and efficiently, which will significantly increase the delivery efficiency and bioavailability of loaded drugs [60]. In addition, NPs can escape rapidly from the endosome to postpone drug degradation and improve its half-life [61]. Furthermore, NPs facilitate prolonged and controllable drug release, which is crucial in the case of adhesion prevention as this process can last many weeks to months [62, 63]. For example, Zhou et al. loaded the TGF-β1 miRNA plasmid into polylactic-co-glycolic acid (PLGA) NPs by double emulsion solvent evaporation technique and successfully achieve sustained gene delivery

for 4 weeks (**Figure 3A**). By specifically reducing the TGF-β1 gene expression, the adhesion formation in the tendon defect site could be significantly reduced after 6 weeks of treatment [64]. However, since tendon repair is a long process, further evaluation of the *in vivo* long-term efficacy (more than 3 months) of the NP-based gene delivery platforms should be conducted. Moreover, although these NP systems showed minimal invasive (local injection), extended drug action time and reduced toxicity, they could not act as a physical barrier to physically or spatially separate the tendon from the surrounding tissue and prevent the migration of extrinsic cells (i.e., fibroblasts) to the injured site, thus gaining partial preclinical success only. To overcome these limitations, various barrier materials like silica gel, gold foil, hydrogels and fibrous membranes have been extensively investigated for post-operative TA prevention. Among them, silica gel and gold foil are outdated and have been gradually eliminated due to their non-degradability and non-permeability [65]. With the development of material science, researchers have shifted their focus to biodegradable polymers including hyaluronic acid (HA), collagen, polyethylene glycol (PEG), PLGA, and poly(lactic acid-co-ethylene glycol-co-lactic acid) (PELA), etc.[19, 66, 67]. Compared to non-absorbable materials, absorbable polymers have the following advantages: (1) They possess good biocompatibility and biodegradability; (2) They have good permeability to allow nutrient transport to local tendon tissues; (3) Some materials like HA, can inhibit inflammatory reactions and collagen secretion, promote intrinsic tendon healing while suppressing the formation of TA [19]. At present, these absorbable polymers have been fabricated into hydrogels or fibrous membranes to work as physical barriers and have achieved a certain degree of success, which will be discussed in the following subsections.

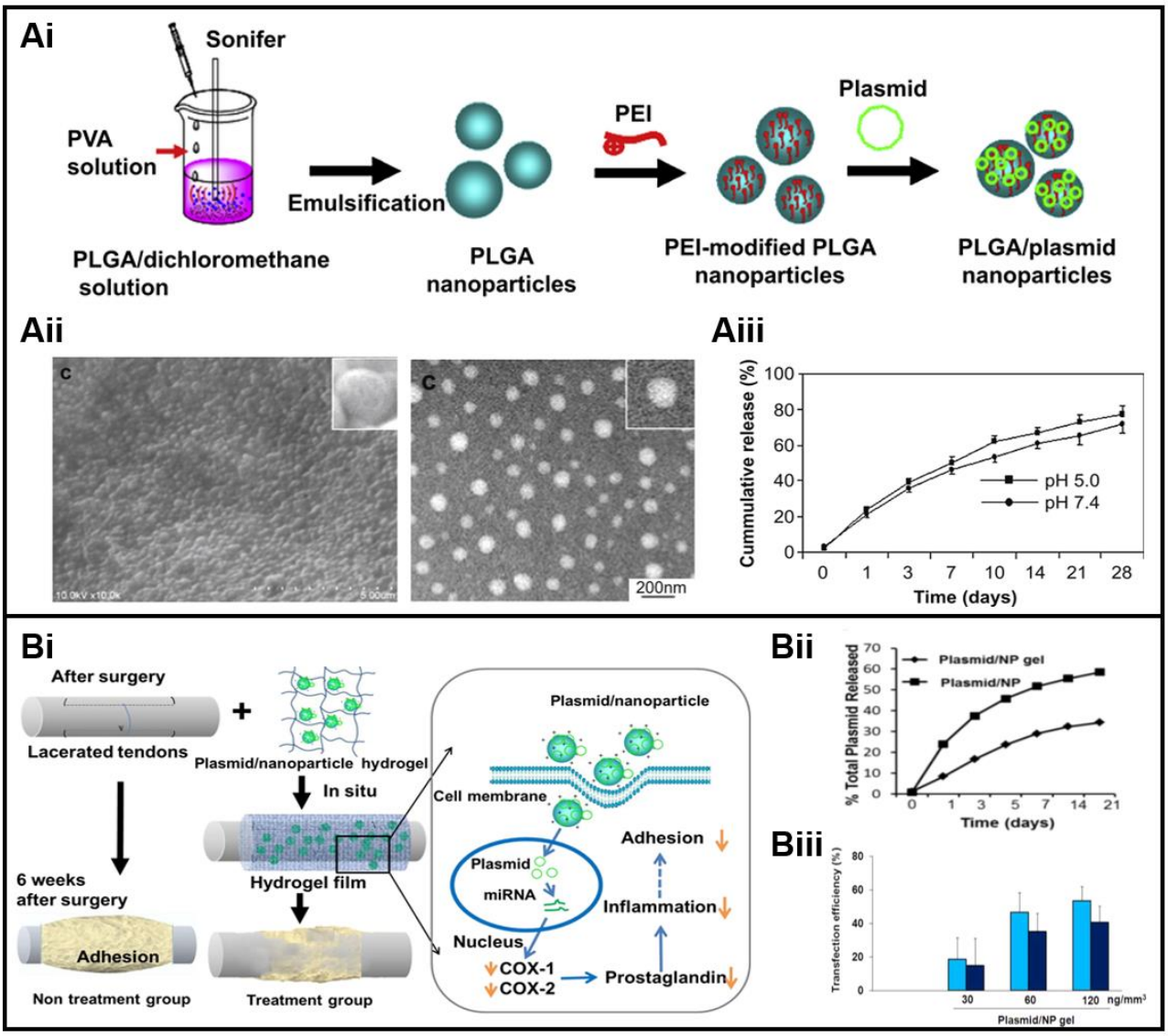

Figure 3. A. Construction of plasmid/PLGA nanoparticle complexes for prevention of tendon adhesion. (Ai) Schematic showing the synthesis process of TGF-β1 miRNA plasmid/PLGA nanoparticle complexes. (Aii) SEM and TEM images of plasmid/PLGA nanoparticle complexes. (Aiii) The *in vitro* release pattern of plasmid from the plasmid/ PLGA nanoparticle complexes. Reproduced from ref. [64] with permission from Elsevier. B. Construction of plasmid/PLGA complex-embedded hydrogel scaffolds to reduce the adhesion formation. (Bi) Schematic showing the underlying mechanism of plasmid/PLGA complex-embedded hydrogel scaffolds for adhesion prevention. (Bii) *In vitro* release pattern of plasmid from the PLGA/plasmid complexes and the PLGA/plasmid nanoparticle-embedded hydrogel scaffolds. (Biii) *In vivo* transfection efficiency of hydrogel scaffolds loaded with different doses of PLGA/plasmid complexes. Reproduced from ref. [74] with permission from Elsevier.

4.2 Hydrogels

As a unique class of polymeric materials, hydrogels with a three-dimensional (3D) network

structure have been widely used in tendon repair and regeneration [14]. Due to their structural porosity, high water content, and similarity to cell and tissue environments, the hydrogel-based scaffolds exhibit favorable biocompatibility and can be served not only as a local niche to control cellular growth and differentiation, facilitating tendon-like tissue growth, but also as an anti-adhesion shield to prevent scar tissue formation [68]. In addition, by varying concentration and crosslinking levels, the hydrogel's physiochemical properties such as degradation rate and mechanical strength can be readily manipulated to meet different application requirements [5]. Among different hydrogel-based materials, injectable hydrogels show great potential since they can gel *in situ* post-injection and allow adequate wrapping around the complicated tendon defect sites to prevent post-operative adhesion [69, 70]. Compared with rigid barrier films like gold foil, such injectable hydrogels are more flexible in practical applications, especially suitable for tendon repair with irregularly shaped defects [71]. Moreover, this injection-based therapy is more convenient and cost-effective than traditional open surgery, representing a minimal invasive manner to reduce patient suffering. In one study, Chou et al, designed a thermo-responsive *in-situ* forming hydrogel by grafting the poly(N-isopropylacrylamide) with chitosan and HA [72]. Due to the cytostatic effects of chitosan, such injectable hydrogel could significantly suppress fibroblast growth and penetration *in vitro*. Additionally, animal experiments further indicated this injectable hydrogel could work as a biodegradable physical barrier to reduce post-operative peritendinous adhesion without hampering normal tendon repair. However, relying solely on the physiochemical properties of barrier materials to prevent TA is often unsatisfactory, and the incorporation of various drugs or genes into hydrogel systems has thus been an alternative solution to obtain a potent anti-adhesion product [73, 74]. For instance, Zhou et al. constructed a novel gene delivery platform by encapsulating cyclooxygenase-engineered miRNA plasmid-loaded PLGA NPs into HA hydrogel (**Figure 3B**) [74]. This hydrogel scaffold could not only avoid the undesired loss of plasmid during the transplantation into the target site, but also allow for extended plasmid release to alleviate early inflammatory response of the injured tendon, thus remarkably decreasing adhesion formation and increasing tendon healing strength.

Although drug or biomolecule delivery-based hydrogel systems have attracted significant attention for tendon regeneration, they cannot mimic both structure and function of native tendon sheath that consists of an outer fibrotic layer for adhesion suppression and an inner synovial layer for tendon gliding and cell proliferation. To achieve this goal, Chen et al. developed a chitosan-based dual-layer asymmetric hydrogel scaffold by a self-deposition

technique [75]. In their designed scaffold, the smooth and dense membrane structure was used as outer layer to provide mechanical support and obstruct extrinsic tissue ingrowth, while the loose spongy layer seeded with tendon stem/progenitor cells was served as inner layer to offer large amounts of tendon-derived stem cells for intrinsic tendon healing. As a crucial component to maintain the integrity and function of the musculoskeletal system, native tendon tissue exhibits superior mechanical strength to connect muscle to bone and convert forces between them, ensuring body movement and force exertion. Besides, previous studies have demonstrated that higher matrix stiffness and mechanical stimulation (e.g., stretching) could promote the proliferation and tenogenic differentiation of stem cells (e.g., MSCs or TSPCs) by activation of focal adhesion kinase (FAK) or ERK1/2 [76-79]. However, due to the inherent lack of mechanical strength and stiffness, most of the current hydrogel-based scaffolds cannot provide a biomimetic mechanical microenvironment for tendon repair and a sufficient mechanical support over a completely transected tendon before enough host neotissue regenerates [14]. Hence, further development of novel hydrogels with high flexibility and elasticity is highly desired for clinical application in TA prevention.

4.3 Fibrous membranes

Currently, fibrous membranes are the most commonly used scaffolds for tendon repair since they can closely recapitulate the subtle 3D fibrous organization of native tendon ECM [80, 81]. In recent years, various fiber-based technologies like electrospinning [82], wet/jet spinning [83, 84], 3D printing[85], counter-rotating extrusion [86], melt electrowriting [87] and textile approaches [88] have been developed to fabricate fibrous membranes with their fiber diameters ranging from nano- to millimeter- scale. Amongst these existing technologies, electrospinning has been broadly recognized as a facile, straightforward, and versatile technique that can constantly generate nanofibers from a viscoelastic polymer solution driven by electrostatic force [89-92]. By regulating different parameters such as solution viscosity, applied voltage, collection distance, and flow rate, the fiber diameters of resultant electrospun fibrous membranes (EFMs) can be intricately orchestrated to mimic the topographical features of native tendon sheath [93]. In addition, their ultra-fine and tunable porous microstructure also help prevent fibroblast penetration from the extrinsic compartment without hindering nutrient and waste transport in and out of the site of tendon healing, making them an idea physical barrier scaffold [19]. Most importantly, the local microenvironment of tendon healing can be further tuned to reduce inflammation, fibroblast over-proliferation and tendon tissue adhesion by loading different drugs into the EFMs and

controlling the drug release kinetics [18]. For example, they can be functionalized and serve as delivery systems for a myriad of bioactive molecules including pharmacological agents, GFs, and genes. Different techniques of functionalization enable multi-functional and spatiotemporally defined release patterns of concurrently integrated molecules [16]. The comparison of the current commonly used strategies for TA prevention is showed in the **Table 1**. In the following sections, we will introduce the latest applications of EFMs in TA prevention, while discussing in detail the state-of-the-art strategies to produce diverse bioscaffolds with more sophisticated structures by combining electrospinning with different technologies or methods.

Table 1. Therapeutic intervention strategies for TA prevention

Strategies	Advantages	Main drawbacks	Refs
Nanoparticles (NPs)	√ Minimal invasive therapy √ Prolonged drug release	×No physical barrier to prevent extrinsic cell migration	[64]
Hydrogels	√ Local niche for cellular control √ Anti-adhesion shield	× Insufficient load-bearing capacity	[72, 75]
Electrospun fibrous membranes (EFMs)	√ Subtle 3D fibrous microstructure √ Tunable physical property √ Ready functionalization and versatile drug delivery systems	× Uncontrollable and unspecific drug delivery	[18, 19, 64, 105, 106, 112, 115]
NPs + Hydrogels	√ Good physical barriers Avoidance of undesired drug loss	× Cannot mimic structure and function of native tendon	[74]
NPs + EMFs	√ Further physical property modulation √ Protection of fragile biomolecules √ Spatiotemporally controlled drug release	× Unstable electrospinning process	[118, 120, 125]
Hydrogels + EMFs	√ Biomimetic architectures √ Anisotropic mechanical properties	×Redundant fabrication procedure	[79]
Decellularized matrix or amnion	√ Inherent ultrastructure of ECM	× Uncontrollable physiochemical property × Low mechanical performance × Immunological concerns	[126,127, 153, 154]

4.3.1 Effects of EFMs' physical properties on tendon repair

Recently, more studies revealed that the architectural factors of EFMs such as fiber topography and micro-porosity can significantly regulate the cell gene and protein expression by activation of the mechanotransduction pathway during tendon healing [15]. For example, thin membranes consisting of aligned nanofibrous strands mimicking the intrinsic *in vivo* collagen fiber bundles can be prepared via electrospinning technique. These aligned nanofibers exhibit an extremely high surface area and have been shown to influence the cell cytoskeletal organization via focal adhesions, better promoting cell orientation and elongation. In addition, compared to the membranes with randomly-oriented nanofibers, the express of the tendon-related genes are much higher in aligned nanofibrous membranes, which indicated their potential in induction of tenogenic differentiation and commitment [94-97]. The special alignment can also increase the level of integrin α1, α5 and β1 subunits, and myosin IIB of the tendon stem cells to promote the tenogenesis instead of osteogenesis as they share a common signaling pathway to prevent the TA formation [98].

Except for the fiber alignment, some other characteristic of the EFM including the pore size/porosity and fiber diameter may also affect the performance of EFM to prevent TA. Many researchers suggest the loose packing nature of the EFM can allow the fibroblasts infiltrate the barrier through some large pores. Even for some pores smaller than the cells, the fibroblasts can push the surrounding fibers aside and hence infiltrate into the scaffold interior [99]. Thus, the porosity of the EFM for TA prevention should be delicately tuned to effectively hinder the fibroblasts invasion and fulfill sufficient nutrients and waste exchange simultaneously [100]. In terms of fiber diameter, Erisken et al. suggested the fibers with smaller diameter (< 500 nm) could stimulate the fibroblasts for proliferation and matrix deposition with increased TA tendency [101]. On the contrary, the expression of the tendon/ligament transcription factor with larger diameter EFM (> 500 nm) such as decorin, scleraxis and tenomodulin was promoted inducing cells to maintain the fibroblastic phenotype and may prevent the TA formation [101, 102]. Similar results were reported by Lee et al. that the nanoscale fibers induced the initial and proliferative phase of wound repair (e.g., enhanced cell proliferation and matrix deposition synthesis), while the micron-size fibers elicited the remodeling phase of tissue repair (e.g., improved cell organization/adhesion and suppressed cell growth/biosynthesis) [103]. More importantly, they found that fiber alignment could reverse effects of nanoscale fibers to alter fibroblast response from repair to healing, which was a more crucial matrix cue for avoiding scar

formation and promoting tissue healing. Notably, fiber diameter also plays a critical role in tenogenic differentiation and immunomodulation of stem cells, offer a promising avenue for promoting intrinsic healing process [104]. Specifically, scaffolds with small fiber diameter could facilitate differentiation of amniotic epithelial stem cell towards the tenogenic lineage while increasing expressions of pro-regenerative and anti-inflammatory cytokines.

As known, friction is an important factor in tendon regeneration and ultimately adhesion formation. A lubricant surface of the designed scaffold can alleviate the friction between the intrinsic and extrinsic compartments to attenuate the TA formation. Ideally, to facilitate healing, the wounded tendon should move under minimum load necessary to achieve maximum free motion [105]. However, smooth tendon gliding is often impossible after injury as joint stiffness caused by swelling and physical irregularities at the repair site increases the friction between the tendon and the tendon sheath. Failure to establish friction-free motion leads to TA, which represents a further barrier to complete regeneration. Effective ways to reduce the friction including the incorporation materials such as 5-fluorouracil and hyaluronic acid (HA) with low friction efficiency towards the surrounding ECM or the specific materials (e.g., Beeswax, poly(2-methacryloxyethylphosphorylcholine)) that could lower the protein absorption on the EFM surface and thus succumb to lesser tissue adhesion with the ECM [106-109]. Several studies attempted to minimize TA by using low friction sutures containing the joint lubricant and wrapping the sutured tendon in a sheath made out of soft hydrogels like HA until healing was complete [110, 111]. Whilst HA hydrogel-coated tendons healed just as well in terms of mechanical strength compared with tendons treated with commercially available adhesion barriers, the relatively rapid degradation of both types of barriers is likely to do little in terms of longer-term prevention of adhesion formation considering tendon healing can take up to and longer than a year and might even induce an inflammatory reaction exacerbating TA. To this end, poly (l-lactic acid) (PLLA), a biocompatible polyester degrading over a period of 6 months to 1 year (thus resembling the time taken for tendon healing) was electrospun into nanofibrous membranes and layered with a thin lubricating layer of chitosan-collagen and alginate hydrogel [112]. Protein adsorption studies showed lower protein adsorption onto alginate coated membranes due to the lack of protein binding sites in alginate. This, in turn, would help in reducing peritendinous adhesions. As post-production processing of EFMs with hydrogel films is cumbersome, a facile one-step synthesis of PEG/polycaprolactone (PCL) composite EFM was developed [113]. By varying the PEG to PCL ratio, it could be demonstrated that membranes containing 75% PEG were least supportive of fibroblastic adhesion, thus

rendering it a promising lubricant EFM for anti-adhesion after tendon surgery. This was postulated to be due to the active role of PEG in regulating protein adsorption such as fibronectin [114]. By preventing significant fibronectin adhesion, PEG/PCL EFMs containing a relatively high amount of PEG were able to significantly reduce *in vivo* adhesion formation over a period of 8 weeks. Recently, Cheng et al. presented a novel strategy to enhance the lubrication of PCL electrospun membranes by grafting poly (2-methacryloyloxyethyl phosphorylcholine) on their surface [115]. This simple one-step dip coating approach endowed the EFMs with a stale hydrated lubrication layer, which can prevent fibroblast adhesion and proliferation *in vitro* and enhance the anti-adhesion efficacy *in vivo* (**Figure 4A**). In addition to different materials incorporation or surface chemical modification, appropriate physical structure design for EFMs could also reduce the friction and promote tendon gliding. For example, Wang et al. fabricated two layers of PLLA membranes by electrospinning and integrated them into a single layer using a shearing force [116]. Such dual-layer scaffolds could form an artificial space during *in vivo* degradation process to promote tendon gliding, which was beneficial for suppressing peritendinous adhesion and facilitating tendon healing (**Figure 4B**).

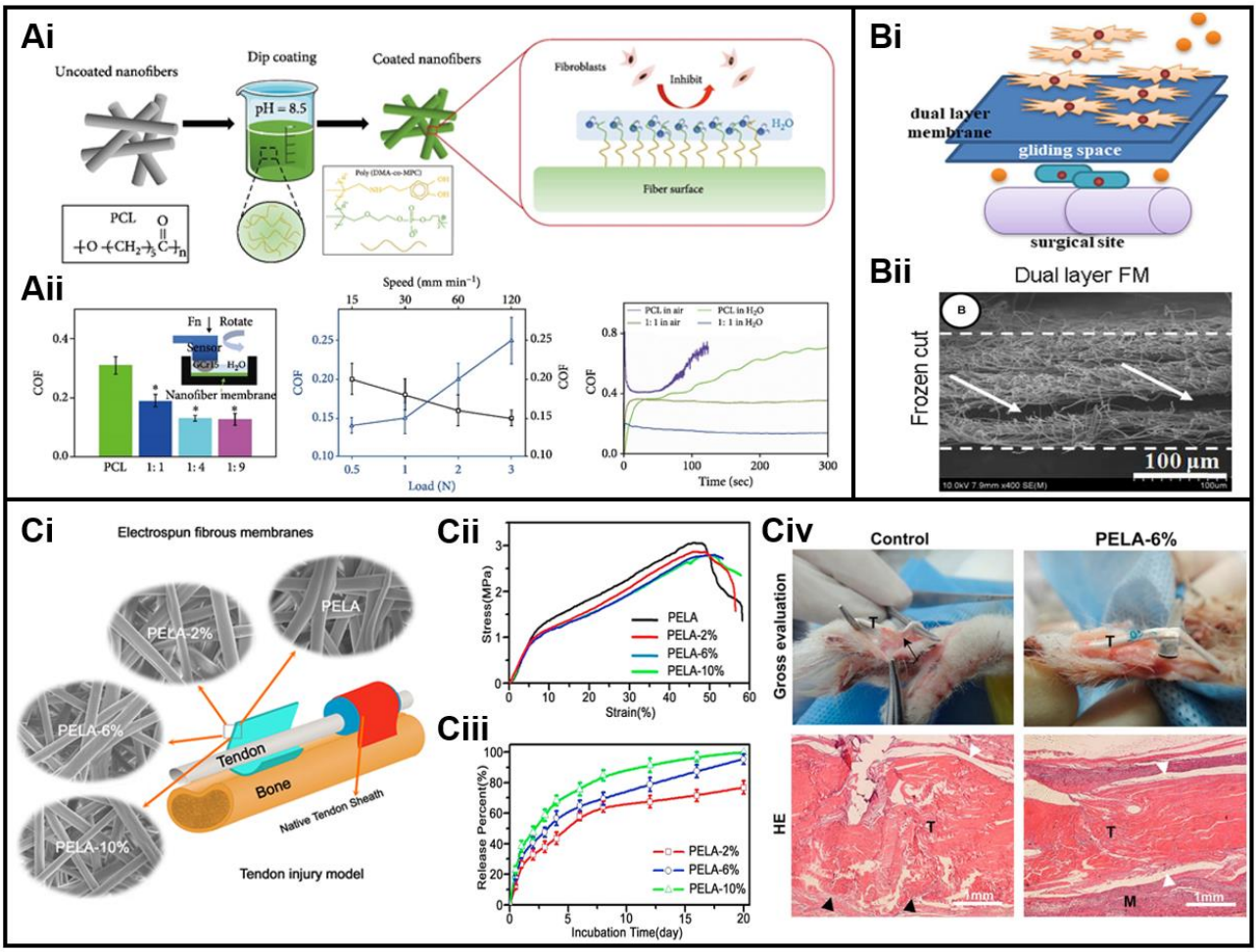

Figure 4: A. Construction of PCL EFMs with a hydrated lubrication layer to prevent tendon

adhesion. (Ai) Schematic showing one-step dip-coating method to graft the poly (2-methacryloyloxyethyl phosphorylcholine) on the surface of PCL EFMs. (Aii) Lubrication tests for the EFMs samples under different conditions. Reproduced from ref. [115] with permission from the Science Partner Journal program. B. Construction of a dual-layer PLLA EFMs for preventing peritendinous adhesion by improving tendon gliding. (Bi) Schematic showing the anti-tendon adhesion mechanism of the dual-layer PLLA EFMs. (Bii) SEM images of dual-layer PLLA EFMs. Reproduced from ref. [116] with permission from Frontiers publisher. C. Construction of a celecoxib-loaded PELA EFMs as drug delivery system and physical barrier for tendon adhesion prevention. (Ci) Schematic showing the fixation of celecoxib-loaded PELA EFMs at injury sit for tendon repair. (Cii) Tensile properties of different PELA EFMs. (Ciii) *In vitro* release profile of celecoxib from different PELA EFMs. (Civ) Gross evaluation and hematoxylin & eosin (HE) staining of repair sites without any treatment (control) or treated with celecoxib-loaded PELA EFMs. Reproduced from ref. [18] with permission from Elsevier.

Altogether, these previous studies provided insights into the importance of controlling EFMs' physical cues in balancing the intrinsic and extrinsic healing process. A proper selection of these physical factors when designing novel scaffolds would lead to an effective promotion of EFM to stimulate extrinsic healing and prevent TA. However, chemotactic factors released from fibroblasts retain the capacity to attract inflammatory cells, such that TA formation can still occur as a result of tissue inflammation. Regulating this inflammatory cascade is thus of principal importance in combating TA as providing a mere physical barrier or physical cues do not sufficiently address the underlying motor of adhesion formation.

4.3.2 EFMs incorporating pharmacological agents

Pharmacological agents including NSAIDs and chemotherapeutics can reduce TA formation by regulating the healing microenvironments. NSAIDs are thought to act through their anti-inflammatory, whilst chemotherapeutics have been shown to reduce fibroblast proliferation and induce their apoptosis [117, 118]. For instance, NSAIDs such as ibuprofen and celecoxib loaded into PELA EFMs were shown to effectively reduce inflammatory response and subsequent TA formation [18, 67]. The ibuprofen could be sustainably released from the EFMs for over 3 weeks and significantly compress the proliferation of the fibroblasts. Both the COX-1 and COX-2, responsible for prostanoid synthesis, are inhibited, resulting in the alleviation of the inflammatory cell infiltration, finally tune the extrinsic healing process [67]. In another study, the celecoxib was load in PELA EFMs and presented to decrease focal

deposition of collagen I and III and inhibit fibroblast adhesion and proliferation (**Figure 4C**). The underlined mechanism may attribute to (1) attenuated ERK1/2 phosphorylation in the MAPK pathway leading to the reduced fibroblast proliferation and (2) attenuated SMAD2/3 phosphorylation in the SMAD pathway inhibiting collagen synthesis [18]. It is worth noting that the regulation of macrophage behaviors by material cues (e.g., surface chemistry, mechanical properties, and topography) or drugs (e.g., ibuprofen) can also reduce inflammatory response, alleviate peritendinous adhesion and improve the accrual of strength during tendon healing. For example, ibuprofen-loaded polylactide EFMs can suppress adhesion, proliferation, and infiltration of macrophages, by blocking the expression of Cox gene, and then inhibit TNF-α expression and collagen III deposition, thereby alleviating inflammation and granuloma formation around the tendon [119]. Moreover, since the activation of M1 macrophages can produce a larger amount of IL-1, TNF-α and IFN-γ and cause local inflammatory response and cell apoptosis, the promotion of macrophage polarization towards the regenerative M2 phenotype has also been demonstrated to facilitate tendon healing [25, 26, 120, 121].

Considering the inconformity of the intrinsic and extrinsic healing, subsequent work using micro-sol electrospinning technique to fabricate core-shell structured EFMs has been put forward to enable spatiotemporal control of drug release kinetics [19]. The loaded chemotherapeutic mitomycin presented initial burst release to prevent fibroblasts proliferation and adhesion formation in the early stage (extrinsic healing) and subsequent steadier release for over 40 days with rarely negative effect on natural tendon healing process (intrinsic healing) (**Figure 5A**). The mitomycin could stimulate exogenous cell apoptosis by up-regulating Bax protein expression whilst down-regulating the Bcl-2, collagen I, collagen III and a-SMA expression [19]. The functionalization of EFMs with various pharmacological agents has provided a new dimension to the treatment and prevention of TA by modulating the behaviors of fibroblasts and attenuating the inflammatory response associated with tendon injury and healing by intervening the signaling pathways of inflammation cytokines and neovascularization. The limitations common to these therapeutic agents remains their relative lack in actively promoting the intrinsic healing process. The following section will discuss the incorporation of GFs or genes into EFMs to stimulate targeted tendon regeneration without eliciting adhesion formation.

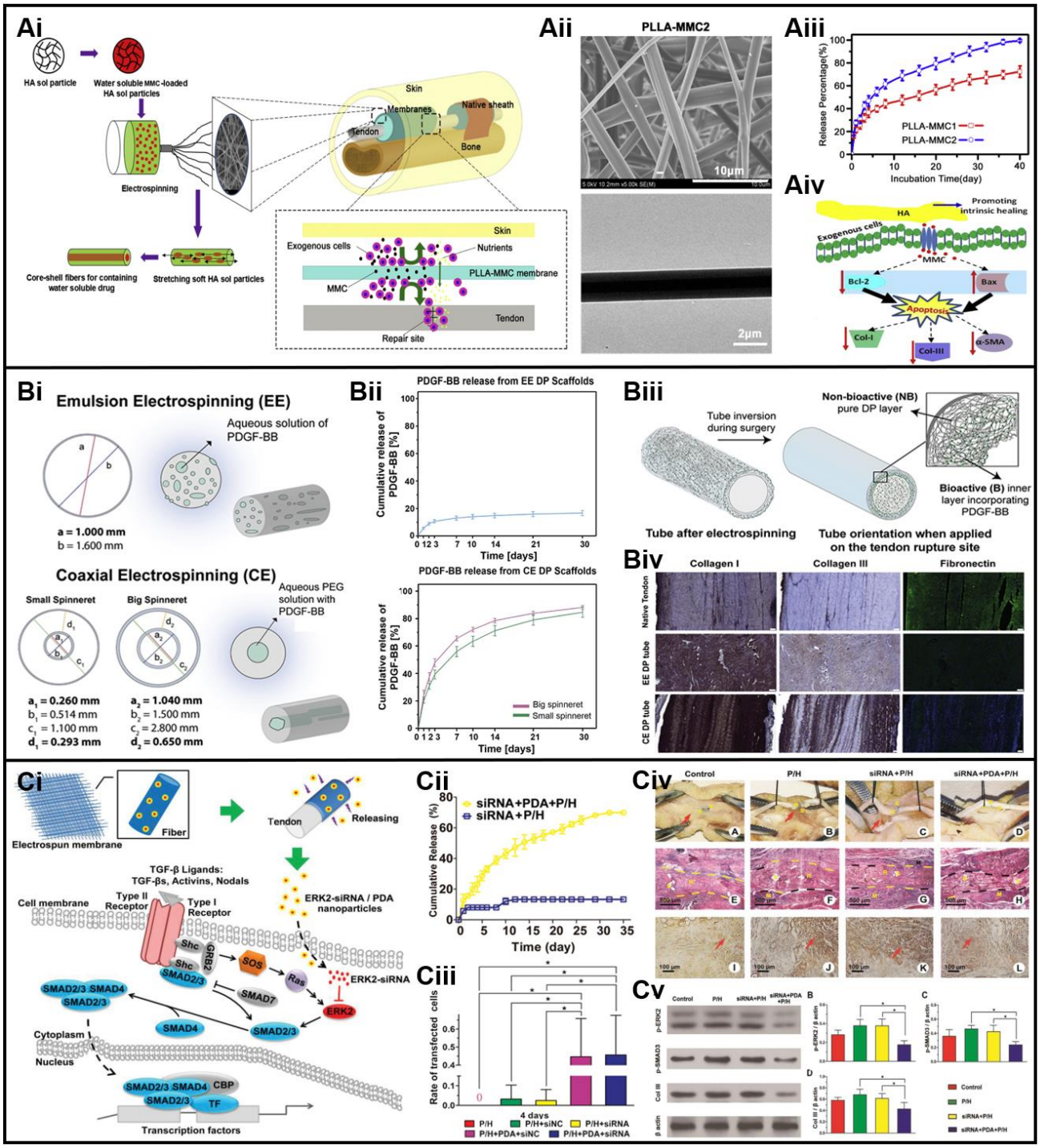

Figure 5. A. Construction of mitomycin-loaded PLLA EFMs for preventing tendon adhesion. (Ai) Schematic showing the fabrication process of mitomycin-loaded PLLA EFMs by micro-sol electrospinning. (Aii) SEM and TEM images of mitomycin-loaded PLLA EFMs. (Aiii) *In vitro* release prolife of mitomycin from EFMs. (Aiv) Proposed mechanism for anti-adhesion by mitomycin-loaded PLLA EFMs. Reproduced from ref. [19] with permission from Elsevier. B. Construction of PDGF-BB-loaded electrospun DegraPol® fibrous membranes for tendon repair. (Bi) Schematic showing the spinneret design for emulsion electrospinning and coaxial electrospinning. (Bii) *In vitro* release prolife of PDGF-BB from emulsion or coaxial electrospun DegraPol® scaffolds. (Biii) Schematic showing the construction of a dual-layer

tube with the PDGF-BB-loaded DegraPol® EFMs as the bioactive layer and the pure DegraPol® EFMs as the non-bioactive layer. (Biv) Collagen I, Collagen III and fibronectin staining of tendon repair site after treatment with PDGF-BB-loaded emulsion or coaxial electrospun DegraPol® scaffolds, as compared with the native tendon tissue. Reproduced from ref. [125] with permission from Elsevier. C. Construction of ERK2-siRNA-loaded PLLA EFMs for tendon adhesion prevention. (Ci) Schematic showing the mechanism of ERK2-siRNA-loaded PLLA EFMs to block the expressions of ERK2 and its downstream signaling molecules. (Cii) *In vitro* ERK2-siRNA release profile from EFMs. (Ciii) The siRNA transfection rate of cells after co-culture with different EFMs. (Civ) Gross evaluation of peritendinous adhesion after 21-day implantation of different EFMs into the injury sites. (Cv) The protein expression of p-ERK2, p-SMAD3, and Col III determined by western blotting. Reproduced from ref. [128] with permission from Wiley.

4.3.3 EFMs incorporating GFs and genes

GFs are fundamentally important signaling moieties intrinsic to any microenvironment within the body. Their ability to affect cellular behaviors by increasing or decreasing the expressions of target genes renders them indispensable for the process of wound healing and regeneration [16]. Due to being physically very frail and prone to rapid degradation, GF delivery to target sites usually requires a protective delivery vehicle of some sort [122]. For example, the platelet-derived GF-BB (PDGF-BB) has been shown to upregulate the expression of signaling integrins in the tenocyte mitogenesis process including α5β1 and α2β1, and thus play a role in cell proliferation, collagen deposition and the formation of a provisional ECM in the tendon intrinsic healing [123]. To evade the non-specific inactivation of the loaded PDGF-BB and realize long-term release, Evrova et al. reported to use emulsion or coaxial electrospinning techniques to create an external polymer shell as a physical barrier towards the surrounding microenvironment [124, 125]. Such interesting design showed that both emulsion and coaxial electrospun membranes could continuously release GFs over a period of 30 days (**Figure 5B**). Moreover, by further constructing a double-layer structure with GF-loaded inner layer and non-bioactive polymer outer layer, this scaffold could achieve a localized and controlled delivery of GFs at repair site, significantly promoting the mechanical strength of repaired tendon without inducing adhesion formation.

Apart from incorporating specific GFs into EFMs, some studies have adopted tendon-derived ECM or amnion that contain diverse bioactive factors to modify the EFMs [126, 127]. In a recent work by Tu et al., an electrospun membrane with aligned and core-shell structure

were fabricated by coaxial electrospinning with tendon-derived ECM encapsulated in the shell layer [126]. This unique design integrated both bioactive ECM components and parallel orientation topography into the scaffolds. Due to the incorporation of tendon-derived ECM, this scaffold could provide an optimal niche environment for MSC recruitment and tenogenic differentiation, effectively promoting the tendon regeneration in an intrinsic manner. In another study, a multilayer composite membrane was prepared by electrospinning PCL nanofibers onto the two surfaces of freeze-dried amnion [127]. This system allowed for sustained release of GFs like TGF-β1, FGF-β, VEGF, and PDGF to activate the ERK1/2 and SMAD2/3 pathways, thereby enhancing intrinsic tendon healing while inhibiting the exogenous adhesion tissue ingrowth. Indeed, the use of these ECM or amnion-based GF reservoirs to promote tendon repair can synergistically amplify the therapeutic efficacy; however, it is difficult to identify and/or eliminate some particular components that are useless or even harmful for tendon repair because of our limited knowledge and techniques. Therefore, further distinguishing different components in these GF reservoirs and investigating their roles in tendon healing process is still essential.

Recently, more studies demonstrated that the effective inhibition of the extrinsic healing relies on the blocking specific cellular molecular signaling and downregulating corresponding signal pathways during TA formation. Except for the GFs, various gene-based delivery systems have also been proposed to transmit the miRNA or siRNA into cells, genetically modulating the expression of cellular signaling molecules. Compared with GFs, gene-based therapy can improve or prevent specific biological functions on a long-term basis and is a more direct choice in the signaling intervention [16]. As an example, Liu at al. reported an extracellular signal-regulated kinase (ERK)2-siRNA deliver system based on electrospun PLLA/HA membranes which could release bioactive ERK2-siRNA for 30 days (**Figure 5C**). The ERK2-siRNA released could downregulate ERK2 expression and its downstream factor SMAD3 in the TGF-β1 signaling pathways to effetely prevent TA formation, presenting a promising strategy in the tendon treatment [128].

Whilst these studies have demonstrated the feasibility of GF or gene incorporation into EFMs with subsequently improved tendon healing and reduced TA formation, these therapeutic avenues are far from being technically mature and the underlying reasons are manifold: (1) the exact nature, combination and chronology of GF or gene release during tendon healing and adhesion formation are highly complex and not fully understood, making their external manipulation difficult and rather vague, (2) it is technically challenging to

deliver these biomacromolecules in precisely defined spatiotemporal patterns for maximum therapeutic effects, (3) the potential adverse effects due to poorly targeted delivery, and (4) the ever present consideration for the tumorigenic roles of GFs or genes cannot but curb the enthusiasm for exposing the body to a potentially dangerous cocktail of signaling factors. To improve controlled drug delivery to only the intended target site, researchers are further refining existing delivery vehicles. One such approach involved the loading of GFs and genes into NPs, which are subsequently integrated into EFMs. The following section will discuss the current efforts in NP and EFMs-combined delivery of signaling factors as well as the therapeutic effects of NPs themselves in reducing TA formation.

4.3.4 EFMs incorporating NPs

Many studies have demonstrated the advantages of NP-based delivery system including relatively high biological safety and loading capacity, low adverse immune responses, facile fabrication in large amounts, easy modification by grafting with particular ligands or small biomolecules for targeting different cell types [129, 130]. Due to the small dimension, NPs can be readily decorated or incorporated on the surfaces or within the electrospun fibers via simple co-electrospinning to bestow upon EFMs new functionalities [16]. In an exemplary study by Liu et al., PLLA EFMs loaded with FGF-β-encapsulating dextran glassy NPs (DGNs) was prepared by blending electrospinning technology (**Figure 6A**). Compared to the conventional electrospinning, this design could achieve better spatiotemporally controlled release of FGF-β for up to 30 days and higher GFs' bioactivity at the same time [131]. *In vitro* cell studies showed that FGF-β release effectively stimulated angiogenesis, cellular differentiation, migration, proliferation and matrix synthesis whilst *in vivo* studies indicated a significantly enhanced promotion of intrinsic tendon healing ability and anti-adhesion profile. Mesoporous silica NP (MSN) is another interesting inorganic drug delivery vehicle that has attracted considerable attention due to its large surface area, high pore volumes, tunable pore size, biocompatibility and facile functionalization by introducing various chemical groups [132]. Incorporation of MSNs into PLLA EFMs could increase the scaffold strength and significantly lower initial burst release of ibuprofen to 6% in the first 12 hours and increase the release profile to 100 days compared to the direct incorporated group with 46% release in the first 12 hours and 20 days release in total (**Figure 6B**). This modified release kinetics could better meet the tendon healing cycle, further improving the performance of long-term anti-inflammation and anti-adhesion of the drugs [133].

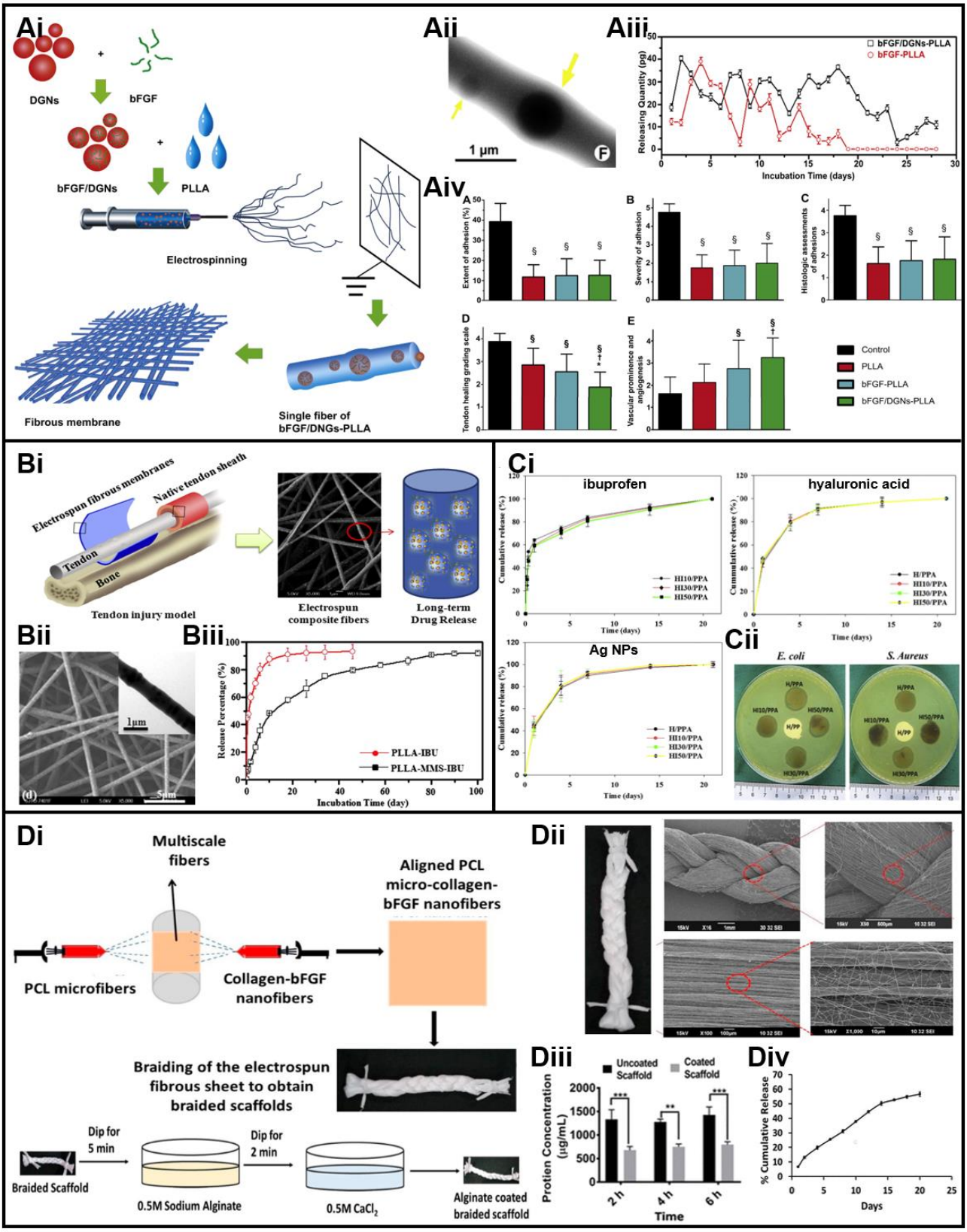

Figure 6. A. Construction of bFGF/DGNs-loaded PLLA EFMs with sustained bFGF bioactivity preservation as anti-adhesion barriers. (Ai) Schematic showing the fabrication process of bFGF/DGNs-loaded PLLA EFMs. (Aii) TEM images of bFGF/DGNs-PLLA EFMs. (Aiii) Release of bFGF from bFGF-PLLA and bFGF/DGNs-PLLA EFMs. (Aiv) Evaluation of the *in vivo* therapeutic efficacy of different EFM samples. Evaluation of the *in vivo* therapeutic

efficacy of different EFM samples for tendon repair. Reproduced from ref. [131] with permission from Elsevier. B. Construction of ibuprofen/MSN-loaded PLLA EFMs with sustained drug release for long-term anti-inflammation and anti-adhesion. (Bi) Schematic showing the composite EFMs as drug carrier and physical barrier for prevention of peritendinous adhesions. (Bii) SEM and TEM images of ibuprofen/MSNs-loaded PLLA EFMs. (Biii) *In vitro* ibuprofen release profile from ibuprofen-loaded PLLA and ibuprofen/MSNs-loaded PLLA EFMs. Reproduced from ref. [133] with permission from Elsevier. C. Construction of ibuprofen and Ag NP-loaded core-shell EFMs for long-term anti-inflammation and antibacteria. (Ci) *In vitro* release profiles of ibuprofen, hyaluronic acid and Ag NPs from core-shell EFMs. (Cii) Antibacterial activity of Ag NPs-containing core-shell EFMs. Reproduced from ref. [138] with permission from Elsevier. D. Construction of braided multiscale fibrous scaffolds for tendon regeneration. (Di) Schematic showing the fabrication process of braided fibrous scaffold by co-electrospinning and subsequent dip-coating. (Dii) Photograph and SEM images of braided multiscale fibrous scaffold. (Diii) Protein adsorption amount on coated and uncoated scaffolds. (Div) *In vitro* bFGF release profile from the braided scaffold. Reproduced from ref. [88] with permission from American Chemical Society.

In addition to being used as delivery vehicles to safely deliver fragile cargoes, NPs like metal NPs have been shown to exert a synergistic anti-adhesion effect during tendon healing. For example, silver NPs (Ag NPs) have emerged as promising NPs for the prevention of TA due to their antibacterial and anti-inflammatory properties [134, 135]. Previous concerns regarding their bulk release-related cytotoxicity were attenuated by constructing multi-functional EFMs capable of releasing incorporated Ag NPs in a controlled and measured fashion. To this end, Chen et al. fabricated HA/PCL EFMs loaded with Ag NPs with a sustained Ag release over 4 days, which is sufficient to prevent bacterial infections during the early post-operative period [136]. Besides, *in vivo* studies impressively showed the positive influence of released Ag on anti-inflammation and adhesion prevention. These results were supported by histological assessments and quantitative evaluations of joint range-of-movement studies. It is believed that the anti-inflammatory effects of Ag were not because of the apoptosis of extrinsic inflammatory cells, but through the effect on the tumor necrosis factor-α (TNF-α) and interferon γ (IFN-γ) which are the most significant pro-inflammatory mediators in immune responses. Through decreasing the expressions of the TNF-α and IFN-γ, some intracellular signaling process in terms of the inflammatory cytokine production, including the nuclear factor-kB(NF-kB) pathway and mitogen-activated protein kinase (MAPK) pathways could be down-regulated, thus to orchestrate the inflammatory

response and TA [137]. In another study, this research group further updated the scaffold design by adding an ibuprofen-laden HA core into Ag NP-containing PEG/PCL shell to create a core-shell structural EFMs (**Figure 6C**) [138]. Such novel barrier membranes could continually release HA, ibuprofen, and Ag for over 3 weeks, which endowed the EFMs with multi-functions like lubrication, anti-infection, and anti-inflammation. Due to their abilities to promote intrinsic tendon healing and relieve extrinsic healing (i.e., TA formation), such scaffolds showed great potential to accommodate the complexity of post-surgical tendon repair. Except for Ag NP, other metal NPs such as gold nanoparticle (Au NP) [139], cupric oxide (CuO) [140] and zinc oxide (ZnO) [141] have also integrated into EFMs to equip the scaffolds with antibacterial activities. Of note, Au NPs may be the most suitable candidate among them to fabricate multifunctional EFMs for tendon repair, since apart from killing bacteria, it can alleviate local inflammation by inhibiting the TNFα and IL-1β expression in the VEGF pathways [142].

4.3.5 EFM-based scaffolds combining with other technologies

Despite the superior ability to produce nanoscale fibers, the electrospinning still faces many challenges like inability to control fiber deposition towards a highly ordered large-scale 3D scaffold, particularly with diameter above 1 mm [14]. To address this issue, hybrid fabrication strategies have been proposed to combine electrospinning with textile technologies like weaving or braiding [143]. Such strategies can not only convert mono- or multi- electrospun fibers into yarns for the construction of well-defined 3D structures, but also further improve the final mechanical properties of the grafts [144]. For example, Jayasree et al. prepared aligned multiscale fibrous sheets consisting of PCL microfibers and collagen-FGF-β nanofibers, and subsequently made them into a braided scaffold using the textile strategy (**Figure 6D**) [88]. Such scaffold design could imitate structural hierarchy and chemical composition (i.e., collagen) of native tendon ECM, and exhibit a significantly improved intrinsic healing. Moreover, the tensile strength of the resultant braided scaffolds (89.4 ± 5.3 MPa) was comparable to the human Achilles tendon [145], facilitating the surgical operation. To further enhance anti-fibrotic ability, this scaffold was then uniformly coated with an alginate layer which has been demonstrated to reduce protein adsorption and cell/fibrous tissue attachment. In another representative study, a woven polydioxanone mesh was first prepared using an industrial loom, and then integrated with a laminated aligned polydioxanone/PCL electrospun membrane in which PCL was used as an adhesive [146]. In this design, one side of the scaffold containing multi-layer polydioxanone electrospun membranes was attached to injured tendon, and the other side (composed of woven

polydioxanone mesh) faced the surrounding tissue. *In vivo* analysis using a non-healing large animal model demonstrated that the electrospun fiber zones offered a good niche for cellular migration and neovascularization, while the woven polydioxanone components prevented exogenous fibroblast infiltration, significantly reducing adhesion formation. Nowadays, electrospinning has also been integrated successfully with other techniques such as rotary jet spinning [83], microfluidics [147], electrospray [148] or 3D printing [149] to construct various scaffolds with ingenious structure and function. An increasing number of anti-tendon adhesion scaffolds based on the combination of these techniques are within reach in the future.

4.4 Other therapeutic platforms

Alongside the general therapeutic structures discussed above, other therapeutic platforms based on different technologies or material components have also been considered potentially useful in inhibiting adhesion formation and promoting tendon healing. For example, Zhou et al. integrated gene-loaded NPs with the commonly used surgical sutures (**Figure 7**) [150]. This gene/NP-tethered sutures could achieve a local and long-term gene delivery over 4 weeks and specifically increase FGF-β and VEGF protein expressions. Further *in vivo* studies indicated that the gene-modified sutures could increase the tendon healing strength by 4-6 times, significantly enhancing the tendon gliding and suppressing adhesion formation, when compared to the unmodified sutures. Another promising strategy to engineer a composite and multi-structural 3D scaffold is to combine electrospun membranes with hydrogels. Rinoldi et al. first fabricated a PCL/nylon-6 nanofibrous membrane by electrospinning, and then deposited a MSCs-laden methacryloyl gelatin/alginate hydrogel thin layer onto the EFMs [79]. The fibrous membranes in the obtained multilayer scaffolds provided biomimetic architectures and anisotropic mechanical properties, while cell-loaded hydrogel layers simulated tendon ECM microenvironment, allowing for cell spreading and proliferation. Due to the low cellularity of native tendon, it is reasonable to assume that the addition of a cell component into the scaffolds can significantly promote the intrinsic healing of damaged tendon tissue. However, traditional cell seeding or encapsulation methods have many limitations such as requirement of high cell number, uneven cell distribution, low cell delivery efficiency. To overcomes these problems, cell-sheet technology has also been adopted to fabricate the stem cell sheet-integrated composite scaffolds [151, 152]. The cell sheets retained the binding motifs on the cell surface and the intact cell-cell interactions, beneficial for reconstruction of tendon rupture. Moreover, decellularized matrix films derived from tendon or cartilage have also

been used directly or combined with other therapeutic platforms to construct biomimetic tendon scaffolds [153, 154]. This strategy could not only capture the inherent ultrastructure of ECM, but also leverage some special anti-adhesive molecules like chondromodulin-1, thrombospondin-1, and endostatin for peritendinous adhesion prevention [153]. Overall, the advance of scaffold fabrication technologies will boost the development of novel therapeutic platforms with improved efficacy and application potential for tendon repair.

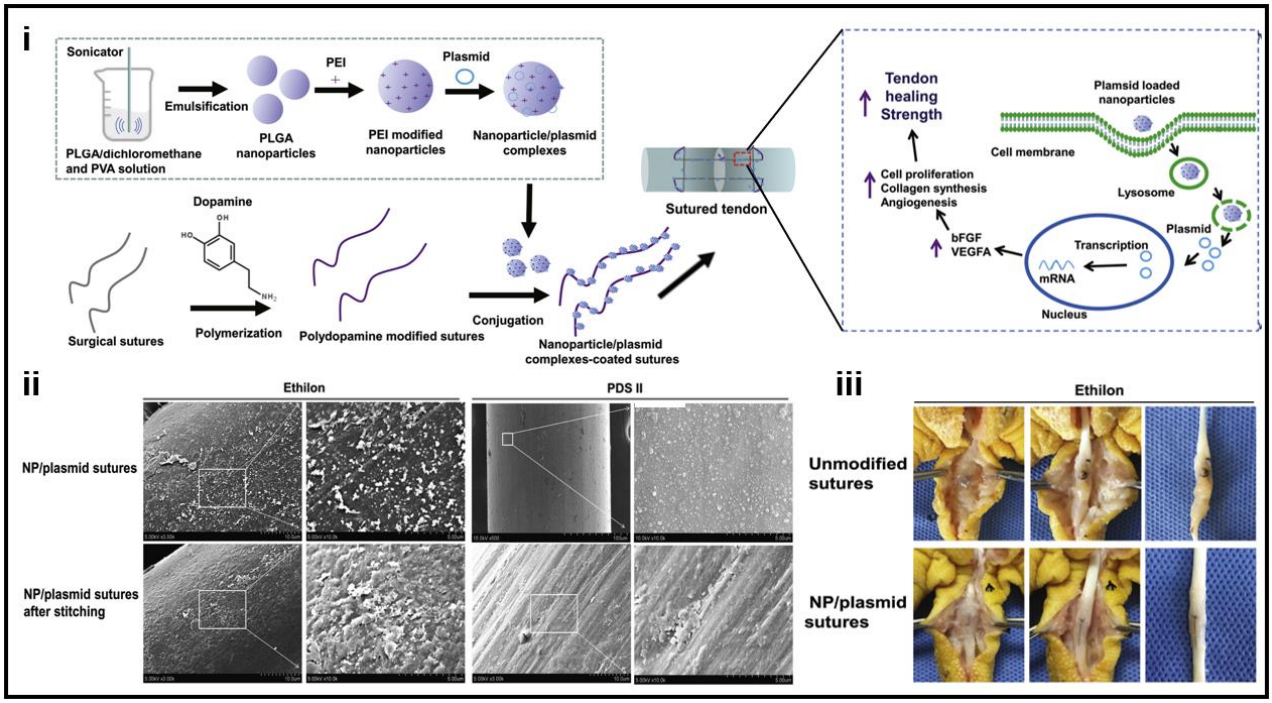

Figure 7. Construction of gene-loaded nanoparticle-coated sutures for tendon healing. (i) Schematic showing the fabrication process of plasmid/nanoparticle complex-coated sutures and their underlying mechanism for tendon repair. (ii) SEM images of Ethilon and PDS II sutures coated with plasmid/nanoparticle complexes before and after stitching. (iii) Therapeutic efficacy evaluation of plasmid/nanoparticle complex-coated sutures for adhesion prevention and tendon healing. Reproduced from ref. [150] with permission from Elsevier.

5. Conclusion and future directions

Tendon injury and repair is commonly complicated by adhesion formation and a reduction or loss in range-of-motion in the affected joint. This can result in a significant economic burden, not to mention hugely impacting on one's quality of life. Thus far, orally administered anti-inflammatory medication and revision operations to release adhesions represent the main therapeutic modalities. In recent years, increasingly sophisticated therapeutic platforms based on NPs, hydrogels or EFMs have emerged as a promising new tool to

prevent adhesion forming in the first place. By material components themselves or incorporation with biomolecules, these therapeutic platforms can effectively regulate the balance between intrinsic and extrinsic healing, significantly enhancing tendon healing while reducing adhesion formation. Amongst these general therapeutic structures, EFMs are a highly promising candidate for tendon repair. They not only provide a physical barrier to TA formation, but also exhibit some features of an ideal drug delivery system: (1) high encapsulation efficiency with retention of bioactive agents' bioactivity; (2) targeting ability into intracellular compartments; (3) biocompatibility, and (4) sustained and modifiable release kinetics. In addition, by turning some physical cues of EFMs like fiber topography, fiber diameter, porosity, or friction performance, an appropriate biological response with improved intrinsic healing and suppressed extrinsic healing can be eventually obtained. Despite such promising advances, several key issues are still waiting to be overcome for the development of EFMs. For example, individual electrospun membranes exhibit relatively weaker mechanical properties as compared to the native tendon tissue, limiting their load-bearing applications; the direct integration of labile biomolecules (e.g., GFs or genes) into EFMs remains challenging due to the involvement of toxic organic solvents or harsh extrusion during the electrospinning. On a positive note, numerous studies have demonstrated that multi-technology or multi-structure combination strategy is a potential solution to address these issues. For instance, strength and stiffness of electrospun nanofibers can be enhanced by using textile technologies like weaving or braiding or combining with microfiber structure. Meanwhile, the addition of labile biomolecules into a protective vehicle (e.g., MSNs) prior to electrospinning or the deposition of a biomolecule-loaded hydrogel layer onto EFMs can remarkably preserve the bioactivity of molecules and improve the biological functions of the obtained grafts.

From a clinical perspective, neither existing or currently researched therapies can fully restore tendon function to pre-injury states and consequently, adhesion and re-ruptures are far too common complications. To design the next-generation therapeutic platforms, some important factors requiring further consideration and the possible solutions are proposed in the following:

(1) First, bioactive agent-loaded scaffolds are currently not able to release drugs in a controllable and desirable manner to match the different stages of tendon repair. A drug delivery system that enables the earlier drug release for inflammatory response regulation and wound healing, and the later extended drug release for adhesion prevention is therefore

highly sought after. In addition, microenvironment-responsive delivery system may be the future avenues of multi-functional scaffold development, whereby the release of active agents is stimulated by changes in the local microenvironment such as changes in pH or GF concentrations. By engineering scaffolds capable of encapsulating agents for many months, even later stage changes in the microenvironment will bring about an adequate release of the desired therapeutic agents.

(2) Traditional physical barriers often fail to deliver drugs to a specific area or one side of the barrier membranes, which may reduce drug effectiveness and cause unsatisfactory side effects. From a structure design's standpoint, a multilayer film scaffold with different drugs incorporated in each layer may be a potential solution to this issue. Specifically, the anti-fibrotic agent-loaded outer layer acts as a barrier film, and the pro-healing agent-loaded inner layer serves as a wounding patch. In addition, when designing the bionic and multi-layer nanofibrous membranes, the interfaces between different layer structures should be considered thoroughly, since the interfaces may play a critical role in the properties (e.g., mechanical property) of the whole scaffolds. However, current research progress on the interface of materials for preventing tendon adhesion is still slow, and thus further exploration is needed.

(3) To date, regulation of the cell microenvironment by inhibiting inflammatory cascade, reducing fibrin deposition, and suppressing fibroblast proliferation, has made some success in preventing the occurrence of adhesion. However, our current knowledge on the native niche and dynamic tendon healing process is limited, thus rendering the exact recapitulation and targeted modulation a sheer impossible task. Further investigation of tendon biology changes during healing, modeling, and remodeling is warranted.

(4) The surgery operation for tendon repair is relatively cumbersome since the implanted scaffolds must be sutured or otherwise attached to tendon tissue. This will prolong the surgical time and increase the risk of postoperative complications. The exploration of multi-material combination by introducing the self-healing or shape memory polymers could deal with this problem. Additionally, due to the discrepancies of tendon injuries in the type (e.g., flexor tendon, Achilles tendon or Hills tendon), location (e.g., tendon itself or bone-tendon interface) and patient's physical condition (e.g., disease or age), a series of grafts with different structures and functions should be developed to meet the anatomical, histological, biochemical, and biomechanical requirements of each individual patient.

(5) The majority of these therapeutic interventions described here have only been studied *in vitro* or in simple animal models, making the extrapolation of results into a clinical setting difficult both in terms of validity and safety. Moreover, these therapeutic platforms do not allow for largescale preparation and off-the-shelf use because of fragile cells or GFs incorporated. Nevertheless, the obtained information from previous studies provides valuable information, which can, in turn, guide further investigations. In the long run, translational studies applying principles gained through *in vitro* experiments in clinically relevant settings will be indispensable to bridge the currently insurmountable knowledge gap. Once clinically applicable scaffolds capable of providing both a physical barrier to adhesion formation and delivering safe and effective dosages of the required composition of bioactive signaling factors have been developed, functional tendon regeneration and minimal or no scar formation is likely to succeed.

Taken together, the successful preparation of anti-adhesion grafts for tendon repair highly rely on the collaborations between experts with different backgrounds, including engineers, material scientists, chemists, biologists, and clinicians. With evermore advanced development of biomaterials and fabrication technologies in conjunction with a better understanding of tendon injury pathophysiology, we believe that a new generation of therapeutic platforms will be established.

Declaration of Competing Interest

The authors declare no conflict of interest.

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